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(54) **Low binding affinity interleukin-12 beta receptors**

(57) The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

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Description

This invention relates generally to Interleukin-12 receptors, especially to human Interleukin-12 receptors.

Interleukin-12 (IL-12), formerly known as cytotoxic lymphocyte maturation factor or natural killer cell stimulatory factor, is a 75-kDa heterodimeric cytokine composed of disulfide-bonded 40-kDa (p40) and 35-kDa (p35) subunits that has multiple biological activities including stimulation of the proliferation of activated T and NK cells (Gately, M. K., et al., 1991, J. Immunol., 147:874) (Kobayashi, M., et al., 1989, J. Exp. Med., 170:827), enhancement of the lytic activity of NK/LAK cells (Kobayashi, M., et al., 1989, J. Exp. Med., 170:827; Stern, A.S., et al., 1990, Proc. Natl. Acad. Sci. USA, 87:6808), enhancement of cytolytic T-cell responses (Gately, M.K., et al., 1992, Cell. Immunology, 143:127), induction of interferon gamma by resting and activated T- and NK-cells (Kobayashi, M. et al., 1989, J. Exp. Med., 170:827; Chan, S. H., et al., 1991, J. Exp. Med., 173:869), and promotion of T_h1-type helper cell responses (Manetti, R., et al., 1993, J. Exp. Med., 177:1199; Hsieh, C.-S., et al., 1993, Science 260:547).

The biological activity of IL-12 is mediated by the binding of the IL-12 molecules to cell surface, or plasma membrane, receptors on activated T-and NK cells; however, the contributions of the individual subunits, p35 and p40, to receptor binding and signal transduction remain unknown. Studies with labeled IL-12 have shown that this binding occurs in a specific and saturable manner. IL-12 delivers a signal to target cells through a receptor that was initially characterised on phytohaemagglutinin (PHA)-activated CD4+ and CD8+ T-cells and on IL-2 activated CD56+ NK-cells (Chizzonite, R., et al., 1992, J. Immunol., 148:3117; Desai, B., et al., 1992, J. Immunol., 148:3125).

A survey of over 20 human cell lines belonging to the T-, B-, NK- and myelomonocytic lineages only identified a single CD4+, IL-2 dependent human T-cell line (Kit 225/K6) that constitutively expresses the IL-12 receptor and responds to IL-12 (Desai, B., et al., 1992, J. Immunol., 148:3125; Desai, B., et al., 1993, J. Immunol. 150:207A). Freshly prepared PHA-activated peripheral blood mononuclear cells (PBMC) and the Kit 225/K6 cell line thus represent two convenient cell sources to study the biochemistry of the functional IL-12 receptor; there may be others.

Equilibrium binding experiments with ^{125}I-labeled IL-12 identified three sites with binding affinities for human IL-12 of 5-20 pM, 50-200 pM, and 2-6 nM on IL-12 responsive T-cells (Chizzonite, R., et al., 1994, Cytokine 6(5):A82a).

A cDNA encoding a low affinity IL-12 receptor was previously cloned (Chua, A., et al, 1994, J. Immunology 153:128; European Patent Application No. 0,638,644). Based on a previously suggested nomenclature (Stahl and Yancopoulos, 1993, Cell 74:587), the initially isolated human IL-12 receptor chain is called the beta1 chain.

The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

Brief description of the drawings:

Figure 1: DNA sequence of human IL-12 receptor beta2 cDNA. (start codon = nucleotide 641; stop codon = nucleotide 3226.)(SEQ ID NO:1).

Figure 2: Amino acid sequence of human IL-12 receptor beta2 protein. (single underlined amino acid residues at the N-terminal sequence = signal peptide; amino acid nos. 623-646 = transmembrane area, marked by double underline; 9 potential N-linked glycosylation sites in the extracellular portion are marked by bold italics and are also underlined; conserved box 1 and 2 motifs in the cytoplasmic domain are shaded [amino acid residues nos. 667-669, 699-704, 786-798])(SEQ ID NO:2).

Figure 3: DNA sequence of human IL-12 receptor beta1 cDNA (start codon = nucleotide 65; stop codon = nucleotide 2050)(SEQ ID NO:3).

Figure 4: Amino acid sequence of human IL-12 receptor beta1 protein. (underlined amino acid residues of N-terminal sequence = signal peptide sequence; amino acid residues nos. 541 to 571 = transmembrane area marked by ------; 6 potential N-linked glycosylation sites in the extracellular portion marked by ------; conserved box 1 and 2 motifs in the cytoplasmic domain are marked by ====== [amino acid residues nos. 577 to 584 and 618 to 629])(SEQ ID NO:4).

Figure 5A: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta1 receptor protein.

Figure 5B: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta2 receptor protein.

Figure 5C: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein.

Figure 6: Analysis of proliferation, in the presence of various concentrations of human IL-12, of Ba/F3 cells stably transfected with cDNA for human IL-12 beta1 receptor protein (-- ♦ --), with cDNA for human IL-12 beta2 receptor protein (-- ○ --), or with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein (-- ● --), by measuring incorporation of tritiated thymidine.

The present invention relates to a low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12. In a preferred embodiment of the present invention the IL-12 beta2 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:2 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:1. In a more preferred embodiment the nucleic acid encoding the IL-12 beta2 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:1. Especially, the invention relates to the human IL-12 beta2 receptor protein having for example the amino acid sequence of SEQ ID NO:2 or allelic forms or variants thereof.

In addition, the invention relates to a complex capable of binding to IL-12 with high affinity, comprising the IL-12 beta2 receptor protein, or a fragment thereof as defined above complexed with human IL-12 beta1 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

In a preferred embodiment the above complex comprises an IL-12 beta1 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:4 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:3. In a more preferred embodiment the nucleic acid encoding the IL-12 beta1 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:3. Especially, the invention relates to the human IL-12 beta1 receptor protein having for example the amino acid sequence of SEQ ID NO:4 or allelic forms or variants thereof.

The present invention also relates to the above proteins or complexes which are soluble.

An aspect of the present invention is a protein or complex encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined above, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region. The invention also includes proteins encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of an IL-12 receptor beta2 protein mentioned above and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a IL-12 receptor beta1 protein and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.

The term "human IL-12 beta2 receptor protein" refers to (1) the protein of SEQ ID NO:2, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:2 and which has the following properties:

1) The protein or polypeptide has low binding affinity for human IL-12, and

2) The protein or polypeptide, when complexed with human beta1 eceptor protein forms a complex having high binding affinity for human IL-12.

The term "human IL-12 beta1 receptor protein" refers to (1) the protein of SEQ ID NO:4, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:4 and which has the following properties:

1) The protein or polypeptide binds to has low binding affinity for human IL-12, and

2) The protein or polypeptide, when complexed with human beta2 receptor protein forms a complex having high binding affinity for human IL-12.

As used herein, the terms "IL-12 beta2 receptor protein" and "IL-12 beta1 receptor protein" includes proteins mod-

ified deliberately, as for example, by site directed mutagenesis or accidentally through mutations. The terms also includes variants which may be prepared from the functional groups occurring as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein and do not confer toxic properties on compositions containing it. These variants may include, for example, polyethylene glycol side-chains which may mask antigenic sites and extend the residence of the proteins in body fluids. Other variants include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl- or threonyl residues) formed with acyl moieties.

"Substantially homologous", which can refer both to nucleic acid and amino acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which do not result in an adverse functional dissimilarity between the reference and subject sequences. For purposes of the present invention, sequences having greater than 80 %, more preferable greater than 90% homology and still more preferably greater than 95% homology, equivalent biological properties, and equivalent expression characteristics are considered substantially homologous. For purposes of determining homology, truncation of the mature sequence should be disregarded. Sequences having lesser degrees of homology, comparable bioactivity, and equivalent expression characteristics are considered substantial equivalents. Generally, homologous DNA sequences can be identified by cross-hybridisation under high stringency hybridisation conditions.

"A fragment of the IL-12 beta2 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of a IL-12 beta2 receptor protein, and which (a) has low binding affinity for IL-12, and (2) when complexed with a IL-12 beta1 receptor protein, forms a complex having high binding affinity for IL-12.

"A fragment of the IL-12 beta1 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of IL-12 beta1 receptor protein, and which when complexed with a IL-12 beta2 receptor protein, forms a complex having high binding affinity for IL-12.

A "soluble fragment" refers to a fragment of a IL-12 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of the protein and which retains the IL-12 binding activity of the intact IL-12 receptor protein. For example, a soluble fragment of a IL-12 beta2 receptor protein is a fragment of a IL-12 beta2 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of a human IL-12 beta2 receptor protein.

In accordance with the invention, a "complex" comprising IL-12 beta2 receptor protein, or a fragment thereof, complexed with IL-12 beta1 receptor protein, or a fragment thereof, may be expressed on the cell surface of the host cell. When expressed on the cell surface of the host cell, the complex has a high binding affinity for IL-12, whereas the IL-12 beta1 receptor protein and the IL-12 beta2 receptor protein alone each have a low binding affinity for IL-12.

In accordance with this invention, the IL-12 beta2 receptor protein may be expressed on the surface of a host cell.

In accordance with this invention, not only the IL-12 beta2 receptor protein may be obtained, but also fragments of IL-12 beta2 receptor protein which (1) have low binding affinity for IL-12 and (2) which when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity. The fragments of IL-12 beta2 receptor protein may be obtained by conventional means, such as (i) proteolytic degradation of the human IL-12 beta2 receptor protein, (ii) chemical synthesis by methods routine in the art, or (iii) standard recombinant methods.

For purposes of the present invention, a human IL-12 receptor protein which has a high binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 5 to about 100 pM. For purposes of the present invention, a human IL-12 receptor protein which has a low binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 1 to about 10 nM. The binding affinity of a protein for IL-12 can be determined by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5.

Fragments of IL-12 beta2 receptor protein can also be measured for binding affinity for IL-12 by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5. The fragments of IL-12 beta2 receptor protein may be measured for binding affinity for IL-12 either alone or complexed with IL-12 beta1 receptor protein, or a fragment of IL-12 beta1 receptor protein which when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity.

The present invention also relates to nucleic acids, e.g. DNA, cDNA, RNA, mRNA, etc. encoding the above proteins, for example a complex capable of binding to human IL-12 with high affinity, the complex comprising human IL-12 beta2 receptor protein, or a fragment thereof, and human IL-12 beta1 receptor protein, or a fragment thereof. Preferably these nucleic acids encode the human IL-12 beta2 receptor protein such as a nucleic acid having the SEQ ID NO:1 and/or the IL-12 beta1 receptor protein such as a nucleic acid having the SEQ ID NO: 3. The present invention also relates to recombinant vectors comprising an above nucleic acid, to expression vectors, and especially to expression vectors wherein the above nucleic acid is operably linked to control sequences recognised by a host cell. The invention includes eukaryotic and prokaryotic host cells transformed with one or more of the above vectors and especially to host

cells wherein the proteins or complexes are expressed on the surface of the host cells and to host cells wherein these cells proliferate in the presence of IL-12. The above host cells may be transformed with a first vector comprising a nucleic acid encoding the IL-12 receptor beta2 protein as defined above and a second vector comprising a nucleic acid encoding the IL-12 receptor beta1 protein as defined above or with a single vector comprising a nucleic acid encoding an IL-12 receptor beta2 protein and a nucleic acid encoding an IL-12 receptor beta1 protein.

As used herein, "nucleic acid" refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from a nucleic acid isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form a DNA or a cDNA with an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

"Expression vector" is a genetic element capable of replication under its own control, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. It comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters and enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences.

"Clone" is a group of identical DNA molecules derived from one original length of DNA sequence and produced by a bacterium or virus using genetic engineering techniques, often involving plasmids.

In addition, the invention refers to a purified, recombinant protein comprising two different polypeptide chains (a heterodimeric protein) which may be prepared by known methods. The two different polypeptide chains are each encoded by a different chimeric polynucleotide which has two nucleic acid subsequences fused in frame. The first nucleic acid subsequence of the first chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of a IL-12 beta2 receptor protein. The second nucleic acid subsequence of the first chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The first nucleic acid subsequence of the second chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of IL-12 beta1 receptor protein. The second nucleic acid subsequence of the second chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region.

The starting materials for the purified, recombinant proteins of the invention may be obtained by methods known in the art. In particular, on the basis of the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 and of the already known nucleic acid sequences for certain receptors, those partial nucleic acid sequences which code for a soluble fragment of IL-12 beta2 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 using known methods, see Sambrook et al., „Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989). Similarly, on the basis of the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 and of the already known DNA sequences for certain receptors, those partial DNA sequences which code for a soluble fragment of human IL-12 beta1 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 using known methods, see Sambrook et al., „Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989). Sources for isolated DNA sequences coding for constant domains of human immunoglobulins are known in the art and disclosed, for example, by Ellison et al., Nucl. Acid Res. 10, 4071-4079 (1982) for IgG_1 or Huck et al., Nucl. Acid Res. 14, 1779-1789 (1986) for IgG_3.

The isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein may be fused in frame, by known methods [Sambrook et al., „Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of the human Ig heavy chain except the first domain of the constant region.

Similarly, the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein may be fused in frame, by known methods [Sambrook et al., „Molecular Cloning", 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of a human Ig heavy chain except the first domain of the constant region.

The chimeric polynucleotides can then be integrated using known methods [Sambrook et al., „Molecular Cloning",

2nd ed., Cold Spring Harbor Laboratory Press (1989)] into suitable expression vectors for expression in a non-human mammalian cell, such as a CHO cell. In order to make the homodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein is integrated into a suitable expression vector. In order to make the heterodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein are integrated into a single suitable expression vector, or two separate suitable expression vectors.

Preferably, the chimeric polynucleotide(s) is/are co-transfected together with a selectable marker, for example neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthin guanine phosphoribosyl transferase (hgpt) using methods which are known in the art. The DNA sequence stably incorporated in the chromosome can subsequently be amplified. A suitable selection marker for this is, for example, dhfr. Mammalian cells, for example, CHO cells, which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transfection has been performed. In this manner, cell lines which contain a higher number of the desired DNA sequence than the unamplified cells can be obtained.

The baculovirus expression system can also be used for the expression of recombinant proteins in insect cells. Postranslational modifications performed by insect cells are very similar to those occurring in mammalian cells. For the production of a recombinant baculovirus which expresses the desired protein a transfer vector is used. A transfer vector is a plasmid which contains the chimeric polynucleotide(s) under the control of a strong promoter, for example, that of the polyhedron gene, surrounded on both sides by viral sequences. The transfer vector is then transfected into the insect cells together with the DNA sequence of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. When using the baculovirus expression system, DNA sequences encoding the immunoglobulin part have to be in the form of cDNA.

The expressed recombinant protein may be purified, for example, by known methods. For example, protein G affinity chromatography may be used to purify the homodimeric protein of the invention. Column chromatography, or any other method that enables differentiation between homodimeric proteins and heterodimeric proteins, may be used to purify the heterodimeric protein of the invention.

Expression of human IL-12 receptor protein having high binding affinity to human IL-12:

The cDNA of cells where the IL-12 receptor is known to be found is incorporated by conventional methods into a bacterial host to establish a cDNA library. PHA-activated PBMC and cells from the Kit 225/K6 cell line are examples of cell sources for the cDNA. RNA from the cells is extracted, characterised, and transcribed into single stranded cDNA by conventional methods. The single stranded cDNA is converted into double stranded cDNA by conventional methods. The double stranded cDNA is incorporated by conventional techniques into an expression vector, such as pEF-BOS. The plasmid DNA from the expression vector is then incorporated into a bacterial host by conventional methods to form a library of recombinants.

The cDNA library is screened by conventional expression screening methods, as described by Hara and Mijayima, 1992, EMBO, 11:1875, for cDNA's which when expressed with cDNA's for the human IL-12 beta1 receptor protein, give rise to a high affinity human IL-12 receptor. A small number of clones from the library are grown in pools. DNA is extracted by conventional methods from the pools of clones. The DNA extracted from a pool of clones is then transfected by conventional methods, along with a small amount of DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein, into non-human host cells. The non-human host cells are preferably mammalian, such as a COS cell. Labeled recombinant human IL-12 is then added to the non-human host cells previously transfected as described above and the binding signal of the pool is determined. This process is repeated for each pool. The pools showing a positive binding signal for IL-12 may then be subsequently broken down into smaller pools and reassayed in the above manner until a single clone is selected which shows a positive binding signal.

The plasmid DNA from the selected clone is sequenced on both strands using conventional methods, such as an ABI automated DNA sequencer in conjunction with a thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. Amino acid sequence alignments may be run as described by M. O. Dayhoff et al., Methods Enzymology 91:524 (1983) with the mutation data matrix, a break penalty of 6 and 100 random runs.

The DNA from the selected clone is then co-transfected by conventional methods with DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

Alternatively, by conventional recombinant methods, a plasmid may be engineered which contains transcription units (promoter, cDNA, and polyA regions) for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Plasmid DNA is transfected by conventional methods into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

In accordance with this invention, DNA may be isolated which encodes human IL-12 beta2 receptor protein, or a

fragment thereof, which fragment (1) has low binding affinity for human IL-12 and (2) when complexed with human IL-12 beta1 receptor protein, forms a complex having high binding affinity for human IL-12.

An isolated nucleic acid sequence refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from nucleic acid isolated at least once in substantially pure form, that is, free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences, e.g. DNA, are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used as a source of coding sequences. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

In accordance with this invention, a mammalian cell having the human IL-12 beta2 receptor protein or the complex expressed on its surface and which proliferates in response to human IL-12 is useful for determining IL-12 bioactivity. For example, such cells are useful for determining whether a given compound inhibits biological activity of human IL-12 or is an IL-12 agonist.

In addition, through the ability to express the human IL-12 beta2 receptor protein on a non-human mammalian cell surface, we can also express fragments of the human IL-12 beta2 receptor protein, and can determine whether these fragments, when complexed with the beta1 subunit, or an active fragment thereof, have the same properties and high binding affinity for IL-12 as the intact complex.

Isolated DNA encoding the human IL-12 beta2 receptor protein may be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein. The isolated DNA encoding the human IL-12 beta2 receptor protein may also be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor protein with the beta2 receptor protein [See, for example, Charnow, S. M. et al., Trends in Biotechnology, Vol. 14, 52-60(1996)].

Such purified, recombinant proteins, which bind to human IL-12, are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors, by inhibiting binding of IL-12 to such cells. Pathological conditions caused by excess activity of cells possessing IL-12 receptors include autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein is the fusion of a soluble fragment of human IL-12 beta2 receptor protein and a human Ig heavy chain (such as IgG, IgM or IgE, preferably IgG) having all domains except the first domain of the constant region. This recombinant protein, which is homodimeric, is encoded by a chimeric polynucleotide which has 2 DNA subsequences fused in frame. The first DNA subsequence, at the 5' end of the chimeric polynucleotide, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence, located at the 3' end of the chimeric polynucleotide, is an isolated DNA sequence encoding all domains of a human heavy chain Ig (preferably IgG) except the first domain of the constant region. The desired recombinant protein can be generated by transfection of the chimeric polynucleotide into a non-human mammalian cell, such as a chinese hamster ovary (CHO) cell. The expressed recombinant protein can be purified, for example, by protein G affinity chromatography.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor with the beta2 receptor is encoded by two chimeric polynucleotides which each have two DNA subsequences fused in frame. The first DNA subsequence of the first chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence of the first chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The first DNA subsequence of the second chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta1 receptor protein. The second DNA subsequence of the second chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The desired recombinant protein may be generated by cotransfection of the two chimeric polynucleotides into a non human mammalian cell, such as a CHO cell. The expressed protein can be purified, for example, by any method that enables differentiation of homodimeric proteins from heterodimeric proteins, such as, for example, column chromatography.

In addition, the invention also relates to a process for the preparation of a protein mentioned above comprising the expression of an above mentioned nucleic acid in a suitable host cell.

In addition, monoclonal or polyclonal antibodies directed against the human IL-12 beta2 receptor protein, or fragments thereof, or the complex, may also be produced by known methods [See, for example, Current Protocols in Immunology, edt. by Coligan, J.E. et al., J. Wiley & Sons (1992)] and used to prevent or treat pathological conditions caused by excess activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

Purified, recombinant proteins are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

„Purified", as used to define the purity of a recombinant protein encoded by the combined DNA sequences described above, or protein compositions thereof, means that the protein or protein composition is substantially free of other proteins of natural or endogenous origin and contains less than about 1% by mass of protein contaminants residual of production processes. Such compositions, however, can contain other proteins added as stabilizers, carders, excipients or co-therapeutics. A protein is purified if it is detectable, for example, as a single protein band in a polyacrylamide gel by silver staining.

Purified recombinant proteins as described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered in clinical treatment of autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis.

The purified recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be used in combination with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF (tumor necrosis factor) receptor, the IL-1 antagonist, and the like to treat or prevent the above disorders or conditions.

In addition, the invention relates to pharmaceutical compositions comprising a protein or an antibody mentioned above and a pharmaceutically acceptable carrier. The pharmaceutical compositions may comprise a therapeutically effective amount of one or more cytokine antagonists.

Further, the invention relates to the use of a protein or an antibody mentioned above for the preparation of a medicament. These compounds are especially useful for the treatment of autoimmune dysfunction.

The dose ranges for the administration of the purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

The dose ranges for the administration of the IL-12 receptor proteins and fragments thereof may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The expected dose range is about 1 ng/kg/day to about 10 mg/kg/day. The IL-12 receptor proteins and fragments thereof can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcohol/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replinishes, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-micorbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

Assays for determining whether a given compound blocks IL-12 activity:

An aspect of the invention is the use of either the human IL-12 beta2 receptor protein or the complex of this invention as a screening agent for pharmaceuticals. In accordance with this invention, we can determine whether a given compound blocks human IL-12 activity or acts as an agonist of IL-12.

A biological activity of human IL-12 is the stimulation of the proliferation of activated T- and NK-cells. Proliferation of activated T-cells causes alloantigen-induced immune responses, such as allograft rejection (such as skin, kidney, and heart transplants) and graft-versus-host reaction in patients who have received bone marrow transplants. This biological activity of human IL-12 is mediated by the binding of the human IL-12 molecules to cell surface receptors on the

activated T-cells.

A compound that blocks human IL-12 activity would, therefore, inhibit the proliferation of activated T-cells and would be useful to treat or prevent alloantigen induced immune responses.

In order to determine if a compound blocks human IL-12 activity, first, a plurality of cells having expressed on their surface either the human IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 and the given compound. Third, it is determined whether the presence of the given compound inhibits proliferation of the cells.

In order to determine if a compound is an agonist of human IL-12, first, a plurality of cells having expressed on their surface either the IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, and which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 or the given compound. Third, it is determined whether the presence of the given compound stimulates proliferation of the cells.

Examples of cells capable of expressing on their surface the complex, which cells proliferate in the presence of human IL-12 include, without limitation, PHA-activated PBMC, Kit 225/K6 cells, and Ba/F3 cells transfected with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Examples of cells capable of expressing on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, which cells proliferate in the presence of human IL-12 include, without limitation, Ba/F3 cells transfected with cDNA for human IL-12 beta2 receptor protein.

In order to determine whether the presence of the given compound inhibits proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells, having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention, are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added either before or simultaneously with human IL-12 to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with human IL-12 and the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly less than the cells of the standard wells, the compound blocks IL-12 activity.

In order to determine whether the presence of the given compound simulates proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly more than cells that were not exposed to human IL-12, the compound is an agonist of human IL-12.

Accordingly, the present invention relates to a method for screening of compounds useful for inhibition of IL-12 activity or compounds useful as agonists of IL-12 activity, comprising contacting a compound suspected of inhibiting IL-12 activity or of being an agonist of IL-12 activity, to a protein mentioned above, followed by detection of the biological effect.

The following examples are offered by way of illustration, not by limitation.

EXAMPLES

MATERIALS AND METHODS:

1. Proteins, Plasmids and Strains

Recombinant human IL-12 (U. Gubler et al., 1991, Proc. Natl. Acad. Sci. USA., 88:4143) was obtained as described therein.

Recombinant human IL-2 (H.W. Lahm et al., 1985, J. Chromatog, 326:357) was obtained as described therein.

The plasmid pEF-BOS is based on a pUC 119 backbone and contains the elongation factor 1 alpha promoter to drive expression of genes inserted at the BstXI site (S. Mizushima and S. Nagata, Nucl. Acids Res., 1990, 18:5322).

The human IL-12 receptor beta1 cDNA in the plasmid pEF-BOS was obtained as described in A. Chua et al., 1994, J. Immunology 153:128 and in European Patent Application Publication No. 0638644.

Electrocompetent *E.coli* DH-10B (S. Grant et al., 1990, Proc. Natl. Acad. Sci USA 87:4645) was obtained from Bethesda Research Laboratory (Bethesda, Maryland).

2. Labeling of Human IL-12 with ^{125}I

Recombinant human IL-12 was labeled with ^{125}I as follows. Iodogen was dissolved in chloroform. 0.05 mg aliquots of Iodogen were dried in 12 x 150 mm borosilicate glass tubes. For radiolabeling, 1.0 mCi Na[^{125}I] was added to the Iodogen-coated borosilicate glass tube, which also contained 0.05 ml of Tris-iodination buffer (25 mM Tris-HCL pH 7.5, 0.4 M NaCl and 1 mM EDTA) to form a ^{125}I solution. The ^{125}I solution was activated by incubating for 6 minutes at room temperature. The activated ^{125}I solution was transferred to a tube containing 0.05 to 0.1 ml recombinant human IL-12 (31.5 mg) in Tris-iodination buffer. The resulting mixture of the activated ^{125}I solution and the recombinant human IL-12 was incubated for 6 minutes at room temperature. At the end of the incubation, 0.05 ml of Iodogen stop buffer (10 mg/ml tyrosine, 10% glycerol in Dulbecco's phosphate buffered saline (PBS), pH 7.40) was added and reacted for 3 minutes. The resulting mixture was then diluted with 1.0 ml Tris-iodination buffer containing 0.25% bovine serum albumin (BSA), and applied to a Bio-Gel P10DG desalting column for chromatography. The column was eluted with Tris-iodination buffer containing 0.25% BSA. 1 ml fractions containing the eluted peak amounts of labeled recombinant human IL-12 were combined. The combined fractions were diluted to $1x10^8$ cpm/ml with 1% BSA in Tris-iodination buffer. Incorporation of ^{125}I into recombinant human IL-12 was monitored by precipitation with trichloroacetic acid (TCA). The TCA precipitable radioactivity (10% TCA final concentration) was typically in excess of 95% of the total radioactivity. The radiospecific activity of the labeled recombinant human IL-12 was typically 1000 to 2000 cpm/fmole.

Example 1

Preparation of Human PHA-activated Lymphoblasts

Human peripheral blood mononuclear cells (PBMC) were isolated from blood collected from healthy donors as described in Gately et al., J. Natl. Cancer Inst. 69, 1245 (1982). The blood was collected into heparinized syringes, diluted with an equal volume of Hank's balanced salt solution and layered over lymphocyte separation medium (LSM® obtained from Organon Teknika Corporation, Durham, North Carolina) in tubes. The tubes were spun at 2000 rpm for 20 minutes at room temperature. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected. Collected PBMC were pelleted at 1500 rpm for 10 minutes through a 15 ml cushion of 20% sucrose in Hank's balanced salt solution. Pelleted PBMC were resuspended in tissue culture medium (1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's medium, supplemented with 0.1 mM nonessential amino acids, 60 mg/ml arginine HCl, 10 mM Hepes buffer, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.05 mM 2-mercaptoethanol, and 1 mg/ml dextrose) (TCM) plus 5% human serum and washed twice in TCM.

The PBMC were then activated to form lymphoblasts. In particular, 0.5 - $1x10^6$ cells/ml in TCM plus 5% human serum plus 0.1% (v/v) PHA-P (Difco, Detroit, MI) were cultured for 3 days at 37°C in a 5% CO_2 atmosphere.

After three days, cell cultures were split 1:1 by volume in TCM plus 5% human serum and 50 U/ml recombinant human IL-2 to yield >95% T-cells. These cells were utilized for preparation of a cDNA library.

Example 2

Extraction and Characterization of RNA

PBMC isolated as in Example 1, activated with PHA for 2-3 days, were harvested and total RNA was extracted using Guanidine Isothiocyanate/Phenol as described by P. Chomczynski and N. Sacchi, Anal. Biochem., 162:156,

1987. PolyA$^+$ RNA was isolated from the total RNA by one batch adsorption to oligo dT latex beads as described (K. Kuribayashi et al., Nucl. Acids Res. Symposium Series 19:61, 1988). The mass yield of this purification was about 4% of polyA+ RNA.

Example 3

cDNA Library

From the above polyA$^+$ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS as follows.

3 mg of polyA$^+$ RNA were reverse transcribed into single stranded cDNAs using RNaseH minus reverse transcriptase in the presence of a-^{32}P-dCTP. The resulting single stranded cDNAs were converted into blunt ended double stranded cDNAs as described by U. Gubler and A. Chua, Essential Molecular Biology Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991. BstXI linkers (A. Aruffo and B. Seed, Proc. Natl. Acad. Sci (USA) 84, 8573, 1987) were ligated to the resulting double stranded cDNAs.

cDNA molecules having a size of greater than 800 base pairs (bp) were selected by size exclusion chromatography as follows. A Sephacryl SF 500 column (0.8 x 29 cm) was packed by gravity in 10 mM Tris-HCl pH 7.8 - 1 mM EDTA - 100 mM NaAcetate. The radioactive cDNA with added BstXI linkers was applied to the column and 0.5 ml fractions were collected. The size distribution of radioactive cDNA was determined by performing electrophoresis on a small aliquot of each fraction on a 1% agarose gel, drying the gel, and visualizing the size by exposure of the gel to X-ray film. cDNA molecules larger than 800 bp were size selected in this fashion.

The selected cDNA molecules were pooled and concentrated by ethanol precipitation. The pooled and concentrated selected cDNA molecules were subsequently ligated to the plasmid pEF-BOS as follows. The plasmid had been restricted with BstXI and purified over two consecutive 1% agarose gels. 300 ng of the restricted and purified plasmid DNA were ligated to 30 ng of size selected cDNA in 60 ml of ligation buffer (50 mM Tris-HCl pH 7.8 - 10 mM $MgCl_2$ - 10 mM DTT - 1 mM rATP - 25 mg/ml BSA) at 15°C overnight.

The following day, the plasmid ligated with the size selected cDNA was extracted with phenol. 6 mg of mussel glycogen were added to the resulting extract, and the nucleic acids were precipitated by ethanol. The resulting precipitate was dissolved in water and the nucleic acids again were precipitated by ethanol, followed by a wash with 80% ethanol. A pellet was formed from the precipitated and washed nucleic acids. The pellet was dissolved in 6 ml of water. 1 ml aliquots of the dissolved pellet were subsequently electroporated into *E.Coli* strain DH-10B. Upon electroporation of 5 parallel aliquots, a library of about 10 million recombinants was generated.

Example 4

Expression Screening for cDNAs Encoding High Affinity IL-12 Receptors

The library was screened according to the general expression screening method described by Hara and Miyajima, 1992, EMBO, 11:1875.

Pools of about 100 E.coli clones from the above library were grown and the plasmid DNA was extracted from the pools by conventional methods. 2 x 10^5 COS cells were plated per 35 mm culture well. COS cells were transfected with a transfection cocktail using the standard DEAE dextran technique described in "Molecular Cloning, a Laboratory Manual", 2nd Ed., J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989 ("Molecular Cloning"). The transfection cocktail contained (1) 1 mg of plasmid DNA extracted from the *E.Coli* clone pools derived from the above library, and (2) 0.1 mg of pEF-BOS plasmid DNA containing the human IL-12 receptor beta1 cDNA.

3 days after transfection, the wells of COS cells were incubated with 10 pM labeled human recombinant IL-12 (specific activity = 1000-2000 cpm/fmole) for 90 minutes at room temperature. The labeled human recombinant IL-12 was removed, and the COS cell monolayer was washed for one hour three times with binding buffer (RPMI 1640, 5% fetal bovine serum (FBS), 25 mM HEPES pH 7) to further select for COS cells expressing high affinity IL-12 receptors only (the binding of the IL-12 ligand to the low affinity sites was further reduced because the low affinity sites have a higher dissociation rate). Subsequently, the cell monolayers were lysed and counted in a gamma counter. After screening 440 pools (representing about 44,000 clones), one pool consistently showed a positive binding signal (300 cpm over 100 cpm background). From this pool, a single clone was subsequently isolated by sib-selection. This single clone (B5-10) contained a cDNA insert of about 3 kb that was completely sequenced.

The cDNA insert of clone B5-10 was incomplete with regard to the protein coding region because it did not contain an in-frame stop codon. The cDNA library of Example 3 was rescreened by conventional DNA hybridization techniques with the cDNA insert from clone B5-10, as described in Molecular Cloning and by Grunstein and Hogness, 1975, Proc. Nat. Acad. Sci. USA., 72:3961. Additional clones were thus isolated and then partially sequenced. The nucleotide sequence of one clone (No. 3) was found to (i) overlap with the 3' end of the nucleotide sequence of clone B5-10, (ii)

extend beyond the nucleotide sequence of clone B5-10, and (iii) contain an in-frame stop codon.

This composite DNA sequence is shown in Figure 1 (SEQ ID NO:1). The deduced amino acid sequence for the encoded receptor protein is shown in Figure 2. Based on the previously suggested nomenclature of Stahl and Yancopolous, 1993, Cell 74:587, we call this newly isolated human IL-12 receptor chain the beta2 chain.

Example 5

Binding Assays

COS cells (4-5x10^7) were transfected by electroporation using a BioRad Gene Pulser (250 mF, 250 volts) with either (1) 25 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein, (2) 25 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein, or (3) a mixture of 12.5 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and 12.5 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein. The electroporated cells were plated in a 600 cm^2 culture plate, harvested after 72 hours by scraping, washed and resuspended in binding buffer.

The cells were assayed to determine affinities of the expressed IL-12 receptors for human IL-12. In particular, equilibrium binding of labeled recombinant human IL-12 to the cells was performed and analyzed as described by R. Chizzonite, et al., 1992, J. Immunol., 148:3117. Electroporated cells (8x10^4) were incubated with increasing concentrations of ^{125}I-labeled recombinant human IL-12 at room temperature for 2 hours. Incubations were carried out in duplicate or triplicate.

Cell bound radioactivity was separated from free labeled ^{125}I-IL-12 by centrifugation of the mixture of electroporated cells and ^{125}I-labeled recombinant human IL-12 through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 (A.H. Thomas) and Silicone Oil AR 200 (Gallard-Schlessinger)) at 4°C for 90 seconds at 10,000 x g to form a cell pellet in a tube. The cell pellet was excised from the tip of the tube in which it was formed, and cell bound radioactivity was determined in a gamma counter.

Receptor binding data were analyzed and the affinities were calculated according to Scatchard using the method described by McPherson, J., 1985, Pharmacol. Methods, 14:213.

Example 6

Production of IL-12 Responsive Cell Line

Wild-type Ba/F3 cells, an IL-3-dependent mouse pro-B cell (Palacios, R. et al., 1985, Cell 41:727) and Ba/F3 cells expressing human IL-12 beta1 receptor protein (Chua, A., et al., 1994, J. Immunology 153:128) were cotransfected with (1) 80 mg of pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and (2) 8 mg of a plasmid expressing a hygromycin resistance gene (Giordano, T.J., et al., 1990, Gene 88:285) by electroporation using a BioRad Gene Pulser (960 mF, 400 volts).

All cells were resuspended at a density of 2 x 10^5 viable cells/ml in a growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and 10% conditioned medium from the WEHI-3 cell line (ATCC No. TIB 68, American Type Culture Collection, Rockville, Maryland). The WEHI-3 cell line is a source of IL-3. The resuspended cells were then incubated at 37°C under 5% CO_2 for 120 hours.

Cells were selected by their ability to grow in (1) the above growth medium in the presence of 1 mg/ml hygromycin or (2) an IL-12 containing growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and various concentrations (10, 50 or 250 ng/ml) of human IL-12.

Ba/F3 cells expressing human IL-12 beta1 receptor protein transfected with pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein grew in the IL-12 containing growth medium, demonstrating that coexpression of human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Additionally, Ba/F3 cells expressing human IL-12 beta2 receptor protein grow in the IL-12 containing growth medium, demonstrating that expression of human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Example 7

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, or (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin G, 100 mg/ml streptomycin, and 2 mM L-glutamine at

2×10^4 cells/well in Costar 3596 flat-bottom microplates for 24 hours. Various dilutions of human IL-12, as shown in Figure 6, were then added to the microplates and the cells were incubated for 42 hours at 37°C in a humidified atmosphere of 5% CO_2 in air. 50 ml of ^{3}H-thymidine, 10 mCi/ml in culture medium, was then added to each well. The cultures were further incubated for 6 hours at 37°C. Subsequently, the culture contents were harvested onto glass fiber filters by means of a cell harvester. ^{3}H-thymidine incorporation was measured by use of a liquid scintillation counter. All samples were assayed in quadruplicate.

Example 8

Sequence Analysis of IL-12 Receptor cDNA Clones and Encoded IL-12 Receptor Protein

The IL-12 beta2 receptor protein, composed of 862 amino acids and a calculated molecular weight of 97231, had the following features: N-terminal signal peptide, extracellular domain, transmembrane domain and cytoplasmic tail. The classical hydrophobic N-terminal signal peptide is predicted to be 23 amino acids in length. Signal peptide cleavage occurs mostly after the amino acids Ala, Ser, Gly, Cys, Thr, Gln (von Heijne, G., 1986, Nucl. Acids Research, 14:4683). For the IL-12 receptor, the cleavage could thus take place after Ala23 in the sequence shown in Figure 2, leaving a mature protein of 839 amino acids based on cleavage at Ala23. The extracellular domain of the receptor is predicted to encompass the region from the C-terminus of the signal peptide to amino acid No. 622 in the sequence shown in Figure 2. Hydrophobicity analysis shows the area from amino acid No. 623 to 646 to be hydrophobic, as would be expected for a transmembrane anchor region. Charged transfer stop residues can be found at the N- as well as the C-terminus of this predicted transmembrane area. The extracellular domain of the receptor is thus 599 amino acids long and contains 9 predicted N-linked glycosylation sites. The cytoplasmic portion is 215 amino acids long (amino acid residue nos. 647 to 862).

Further analysis of the amino acid sequence shown in Figure 2 shows the human IL-12 beta2 receptor protein is a member of the cytokine receptor superfamily, by virtue of the sequence motifs [Cys132 --- Cys143TW] and [W305SKWS]. Comparing the sequence shown in Figure 2 to all the members of the superfamily by running the ALIGN program shows that the human IL-12 beta2 receptor protein has the highest homology to human gp130. The cytoplasmic region of the IL-12 receptor beta2 chain contains the box 1 and 2 motifs found in other cytokine receptor superfamily members, as well as three tyrosine residues. Phosphorylation of tyrosines is commonly associated with cytokine receptor signalling; the presence of these tyrosine residues underscores the importance of the IL-12 receptor beta2 chain in the formation of a functional IL-12 receptor. The IL-12 receptor beta1 chain does not contain any tyrosine residues in its cytoplasmic tail.

Example 9

Analysis of the Binding Assays

The results of the binding assays are shown in Figure 5.

As shown in Figures 5A and 5B, human IL-12 binds to recombinant IL-12 receptor beta1 or beta2 alone with an apparent affinity of about 2-5 nM. The binding data was described by a single site receptor model, corresponding to the low affinity component of the functional IL-12 receptor found on PHA-activated PBMC (R. Chizzonite et al., 1992, J. Immunol., 148:3117; B. Desai et al., 1992, J. Immunol., 148:3125).

In contrast to these results, as shown in Figure 5C, both high and low affinity IL-12 binding sites were generated upon cotransfection of COS cells with IL-12 receptor beta1 and beta2 plasmids. In this case, the binding data were described by a two receptor site model, with affinities of 50 pM and 5 nM.

Example 10

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

The results of the proliferation assay for the effect of human IL-12 on Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, and (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are shown in Figure 6.

Cells that are transfected with cDNAs for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Additionally, cells that are transfected with cDNAs for human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Consequently, isolated cDNA (clone No. B5-10, SEQ.ID. No:1) coding for a type I transmembrane protein represents a second component of the IL-12 receptor (IL-12R beta2) found on normal human T-cells. The beta1 and beta2

chains each alone bind IL-12 only with low affinity (Kd= 2-5 nM). Upon coexpression of beta1 and beta2, two affinity sites are observed, with Kd values of 50 pM and 5 nM.

Ba/F3 cells expressing human IL-12 beta2 receptor protein or coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are responsive to human IL-12.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: HOFFMANN-LA ROCHE AG

(B) STREET: Grenzacherstrasse 124

(C) CITY: Basle

(D) STATE: BS

(E) COUNTRY: Switzerland

(F) POSTAL CODE (ZIP): CH-4002

(G) TELEPHONE: 061-688 51 08

(H) TELEFAX: 061-688 13 95

(I) TELEX: 962292/965542 hlr ch

(ii) TITLE OF INVENTION: RECEPTORS FOR INTERLEUKIN-12

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4040 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 641..3226

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
TGCAGAGAAC AGAGAAAGGA CATCTGCGAG GAAAGTTCCC TGATGGCTGT CAACAAAGTG     60

CCACGTCTCT ATGGCTGTGT ACGCTGAGCA CACGATTTTA TCGCGCCTAT CATATCTTGG    120

TGCATAAACG CACCTCACCT CGGTCAACCC TTGCTCCGTC TTATGAGACA GGCTTTATTA    180

TCCGCATTTT ATATGAGGGG AATCTGACGG TGGAGAGAGA ATTATCTTGC TCAAGGCGAC    240

ACAGCAGAGC CCACAGGTGG CAGAATCCCA CCCGAGCCCG CTTCGACCCG CGGGGTGGAA    300

ACCACGGGCG CCCGCCCGGC TGCGCTTCCA GAGCTGAACT GAGAAGCGAG TCCTCTCCGC    360

CCTGCGGCCA CCGCCCAGCC CCGACCCCCG CCCCGGCCCG ATCCTCACTC GCCGCCAGCT    420

CCCCGCGCCC ACCCCGGAGT TGGTGGCGCA GAGGCGGGAG GCGGAGGCGG GAGGGCGGGC    480

GCTGGCACCG GGAACGCCCG AGCGCCGGCA GAGAGCGCGG AGAGCGCGAC ACGTGCGGCC    540
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CAGAGCACCG GGGCCACCCG GTCCCCGCAG GCCCGGGACC GCGCCCGCTG GCAGGCGACA      600

CGTGGAAGAA TACGGAGTTC TATACCAGAG TTGATTGTTG ATG GCA CAT ACT TTT        655
                                            Met Ala His Thr Phe
                                              1               5

AGA GGA TGC TCA TTG GCA TTT ATG TTT ATA ATC ACG TGG CTG TTG ATT        703
Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile Thr Trp Leu Leu Ile
                 10                  15                  20

AAA GCA AAA ATA GAT GCG TGC AAG AGA GGC GAT GTG ACT GTG AAG CCT        751
Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp Val Thr Val Lys Pro
             25                  30                  35

TCC CAT GTA ATT TTA CTT GGA TCC ACT GTC AAT ATT ACA TGC TCT TTG        799
Ser His Val Ile Leu Leu Gly Ser Thr Val Asn Ile Thr Cys Ser Leu
         40                  45                  50

AAG CCC AGA CAA GGC TGC TTT CAC TAT TCC AGA CGT AAC AAG TTA ATC        847
Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg Arg Asn Lys Leu Ile
     55                  60                  65

CTG TAC AAG TTT GAC AGA AGA ATC AAT TTT CAC CAT GGC CAC TCC CTC        895
Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His His Gly His Ser Leu
 70                  75                  80                  85

AAT TCT CAA GTC ACA GGT CTT CCC CTT GGT ACA ACC TTG TTT GTC TGC        943
Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr Thr Leu Phe Val Cys
                 90                  95                 100

AAA CTG GCC TGT ATC AAT AGT GAT GAA ATT CAA ATA TGT GGA GCA GAG        991
Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln Ile Cys Gly Ala Glu
            105                 110                 115
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ATC TTC GTT GGT GTT GCT CCA GAA CAG CCT CAA AAT TTA TCC TGC ATA       1039
Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln Asn Leu Ser Cys Ile
        120                 125                 130

CAG AAG GGA GAA CAG GGG ACT GTG GCC TGC ACC TGG GAA AGA GGA CGA       1087
Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr Trp Glu Arg Gly Arg
    135                 140                 145

GAC ACC CAC TTA TAC ACT GAG TAT ACT CTA CAG CTA AGT GGA CCA AAA       1135
Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln Leu Ser Gly Pro Lys
150                 155                 160                 165

AAT TTA ACC TGG CAG AAG CAA TGT AAA GAC ATT TAT TGT GAC TAT TTG       1183
Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile Tyr Cys Asp Tyr Leu
                170                 175                 180

GAC TTT GGA ATC AAC CTC ACC CCT GAA TCA CCT GAA TCC AAT TTC ACA       1231
Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro Glu Ser Asn Phe Thr
            185                 190                 195

GCC AAG GTT ACT GCT GTC AAT AGT CTT GGA AGC TCC TCT TCA CTT CCA       1279
Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser Ser Ser Ser Leu Pro
        200                 205                 210

TCC ACA TTC ACA TTC TTG GAC ATA GTG AGG CCT CTT CCT CCG TGG GAC       1327
Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro Leu Pro Pro Trp Asp
    215                 220                 225

ATT AGA ATC AAA TTT CAA AAG GCT TCC GTG AGC AGA TGT ACC CTT TAT       1375
Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser Arg Cys Thr Leu Tyr
230                 235                 240                 245

TGG AGA GAT GAG GGA CTG GTA CTG CTT AAT CGA CTC AGA TAT CGG CCC       1423
Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg Leu Arg Tyr Arg Pro
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            250                 255                 260

AGT AAC AGC AGG CTC TGG AAT ATG GTT AAT GTT ACA AAG GCC AAA GGA    1471
Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val Thr Lys Ala Lys Gly
        265                 270                 275

AGA CAT GAT TTG CTG GAT CTG AAA CCA TTT ACA GAA TAT GAA TTT CAG    1519
Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr Glu Tyr Glu Phe Gln
    280                 285                 290

ATT TCC TCT AAG CTA CAT CTT TAT AAG GGA AGT TGG AGT GAT TGG AGT    1567
Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser Trp Ser Asp Trp Ser
295                 300                 305

GAA TCA TTG AGA GCA CAA ACA CCA GAA GAA GAG CCT ACT GGG ATG TTA    1615
Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu Pro Thr Gly Met Leu
310                 315                 320                 325

GAT GTC TGG TAC ATG AAA CGG CAC ATT GAC TAC AGT AGA CAA CAG ATT    1663
Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr Ser Arg Gln Gln Ile
            330                 335                 340

TCT CTT TTC TGG AAG AAT CTG AGT GTC TCA GAG GCA AGA GGA AAA ATT    1711
Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu Ala Arg Gly Lys Ile
        345                 350                 355

CTC CAC TAT CAG GTG ACC TTG CAG GAG CTG ACA GGA GGG AAA GCC ATG    1759
Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr Gly Gly Lys Ala Met
    360                 365                 370

ACA CAG AAC ATC ACA GGA CAC ACC TCC TGG ACC ACA GTC ATT CCT AGA    1807
Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr Thr Val Ile Pro Arg
375                 380                 385
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ACC GGA AAT TGG GCT GTG GCT GTG TCT GCA GCA AAT TCA AAA GGC AGT      1855
Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala Asn Ser Lys Gly Ser
390                 395                 400                 405

TCT CTG CCC ACT CGT ATT AAC ATA ATG AAC CTG TGT GAG GCA GGG TTG      1903
Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu Cys Glu Ala Gly Leu
                410                 415                 420

CTG GCT CCT CGC CAG GTC TCT GCA AAC TCA GAG GGC ATG GAC AAC ATT      1951
Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu Gly Met Asp Asn Ile
            425                 430                 435

CTC GTG ACT TGG CAG CCT CCC AGG AAA GAT CCC TCT GCT GTT CAG GAG      1999
Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro Ser Ala Val Gln Glu
        440                 445                 450

TAC GTG GTG GAA TGG AGA GAG CTC CAT CCA GGG GGT GAC ACA CAG GTC      2047
Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly Gly Asp Thr Gln Val
    455                 460                 465

CCT CTA AAC TGG CTA CGG AGT CGA CCC TAC AAT GTG TCT GCT CTG ATT      2095
Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn Val Ser Ala Leu Ile
470                 475                 480                 485

TCA GAG AAC ATA AAA TCC TAC ATC TGT TAT GAA ATC CGT GTG TAT GCA      2143
Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu Ile Arg Val Tyr Ala
                490                 495                 500

CTC TCA GGG GAT CAA GGA GGA TGC AGC TCC ATC CTG GGT AAC TCT AAG      2191
Leu Ser Gly Asp Gln Gly Gly Cys Ser Ser Ile Leu Gly Asn Ser Lys
            505                 510                 515

CAC AAA GCA CCA CTG AGT GGC CCC CAC ATT AAT GCC ATC ACA GAG GAA      2239
His Lys Ala Pro Leu Ser Gly Pro His Ile Asn Ala Ile Thr Glu Glu
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        520                     525                     530

AAG GGG AGC ATT TTA ATT TCA TGG AAC AGC ATT CCA GTC CAG GAG CAA      2287
Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile Pro Val Gln Glu Gln
    535                     540                     545

ATG GGC TGC CTC CTC CAT TAT AGG ATA TAC TGG AAG GAA CGG GAC TCC      2335
Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp Lys Glu Arg Asp Ser
550                     555                     560                     565

AAC TCC CAG CCT CAG CTC TGT GAA ATT CCC TAC AGA GTC TCC CAA AAT      2383
Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr Arg Val Ser Gln Asn
                570                     575                     580

TCA CAT CCA ATA AAC AGC CTG CAG CCC CGA GTG ACA TAT GTC CTG TGG      2431
Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val Thr Tyr Val Leu Trp
            585                     590                     595

ATG ACA GCT CTG ACA GCT GCT GGT GAA AGT TCC CAC GGA AAT GAG AGG      2479
Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser His Gly Asn Glu Arg
        600                     605                     610

GAA TTT TGT CTG CAA GGT AAA GCC AAT TGG ATG GCG TTT GTG GCA CCA      2527
Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met Ala Phe Val Ala Pro
    615                     620                     625

AGC ATT TGC ATT GCT ATC ATC ATG GTG GGC ATT TTC TCA ACG CAT TAC      2575
Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile Phe Ser Thr His Tyr
630                     635                     640                     645

TTC CAG CAA AAG GTG TTT GTT CTC CTA GCA GCC CTC AGA CCT CAG TGG      2623
Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala Leu Arg Pro Gln Trp
                650                     655                     660
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TGT AGC AGA GAA ATT CCA GAT CCA GCA AAT AGC ACT TGC GCT AAG AAA    2671
Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser Thr Cys Ala Lys Lys
            665                 670                 675

TAT CCC ATT GCA GAG GAG AAG ACA CAG CTG CCC TTG GAC AGG CTC CTG    2719
Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro Leu Asp Arg Leu Leu
        680                 685                 690

ATA GAC TGG CCC ACG CCT GAA GAT CCT GAA CCG CTG GTC ATC AGT GAA    2767
Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro Leu Val Ile Ser Glu
    695                 700                 705

GTC CTT CAT CAA GTG ACC CCA GTT TTC AGA CAT CCC CCC TGC TCC AAC    2815
Val Leu His Gln Val Thr Pro Val Phe Arg His Pro Pro Cys Ser Asn
710                 715                 720                 725

TGG CCA CAA AGG GAA AAA GGA ATC CAA GGT CAT CAG GCC TCT GAG AAA    2863
Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His Gln Ala Ser Glu Lys
                730                 735                 740

GAC ATG ATG CAC AGT GCC TCA AGC CCA CCA CCT CCA AGA GCT CTC CAA    2911
Asp Met Met His Ser Ala Ser Ser Pro Pro Pro Pro Arg Ala Leu Gln
            745                 750                 755

GCT GAG AGC AGA CAA CTG GTG GAT CTG TAC AAG GTG CTG GAG AGC AGG    2959
Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys Val Leu Glu Ser Arg
        760                 765                 770

GGC TCC GAC CCA AAG CCA GAA AAC CCA GCC TGT CCC TGG ACG GTG CTC    3007
Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys Pro Trp Thr Val Leu
    775                 780                 785

CCA GCA GGT GAC CTT CCC ACC CAT GAT GGC TAC TTA CCC TCC AAC ATA    3055
Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr Leu Pro Ser Asn Ile
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790                 795                 800                 805

GAT GAC CTC CCC TCA CAT GAG GCA CCT CTC GCT GAC TCT CTG GAA GAA     3103
Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala Asp Ser Leu Glu Glu
                810                 815                 820

CTG GAG CCT CAG CAC ATC TCC CTT TCT GTT TTC CCC TCA AGT TCT CTT     3151
Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe Pro Ser Ser Ser Leu
            825                 830                 835

CAC CCA CTC ACC TTC TCC TGT GGT GAT AAG CTG ACT CTG GAT CAG TTA     3199
His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu Thr Leu Asp Gln Leu
        840                 845                 850

AAC ATG AGG TGT GAC TCC CTC ATG CTC TGAGTGGTGA GGCTTCAAGC           3246
Lys Met Arg Cys Asp Ser Leu Met Leu
    855                 860

CTTAAAGTCA GTGTGCCCTC AACCAGCACA GCCTGCCCCA ATTCCCCCAG CCCCTGCTCC   3306

AGCAGCTGTC ATCTCTGGGT GCCACCATCG GTCTGGCTGC AGCTAGAGGA CAGGCAAGCC   3366

AGCTCTGGGG GAGTCTTAGG AACTGGGAGT TGGTCTTCAC TCAGATGCCT CATCTTGCCT   3426

TTCCCAGGGC CTTAAAATTA CATCCTTCAC TGTGTGGACC TAGAGACTCC AACTTGAATT   3486

CCTAGTAACT TTCTTGGTAT GCTGGCCAGA AAGGGAAATG AGGAGGAGAG TAGAAACCAC   3546

AGCTCTTAGT AGTAATGGCA TACAGTCTAG AGGACCATTC ATGCAATGAC TATTTCTAAA   3606

GCACCTGCTA CACAGCAGGC TGTACACAGC AGATCAGTAC TGTTCAACAG AACTTCCTGA   3666

GATGATGGAA ATGTTCTACC TCTGCACTCA CTGTCCAGTA CATTAGACAC TAGGCACATT   3726
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GGCTGTTAAT CACTTGGAAT GTGTTTAGCT TGACTGAGGA ATTAAATTTT GATTGTAAAT 3786

TTAAATCGCC ACACATGGCT AGTGGCTACT GTATTGGAGT GCACAGCTCT AGATGGCTCC 3846

TAGATTATTG AGAGCCTCCA AAACAAATCA ACCTAGTTCT ATAGATGAAG ACATAAAAGA 3906

CACTGGTAAA CACCAATGTA AAAGGGCCCC CAAGGTGGTC ATGACTGGTC TCATTTGCAG 3966

AAGTCTAAGA ATGTACCTTT TTCTGGCCGG GCGTGGTAGC TCATGCCTGT AATCCCAGCA 4026

CTTTGGGAGG CTGA 4040

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 862 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala His Thr Phe Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile
1 5 10 15

Thr Trp Leu Leu Ile Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp
20 25 30

Val Thr Val Lys Pro Ser His Val Ile Leu Leu Gly Ser Thr Val Asn
35 40 45

Ile Thr Cys Ser Leu Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg

50 55 60

Arg Asn Lys Leu Ile Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His
65 70 75 80

His Gly His Ser Leu Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr
85 90 95

Thr Leu Phe Val Cys Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln
100 105 110

Ile Cys Gly Ala Glu Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln
115 120 125

Asn Leu Ser Cys Ile Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr
130 135 140

Trp Glu Arg Gly Arg Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln
145 150 155 160

Leu Ser Gly Pro Lys Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile
165 170 175

Tyr Cys Asp Tyr Leu Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro
180 185 190

Glu Ser Asn Phe Thr Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser
195 200 205

Ser Ser Ser Leu Pro Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro
210 215 220

Leu Pro Pro Trp Asp Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser
225 230 235 240

Arg Cys Thr Leu Tyr Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg
245 250 255

Leu Arg Tyr Arg Pro Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val
260 265 270

Thr Lys Ala Lys Gly Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr
275 280 285

Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser
290 295 300

Trp Ser Asp Trp Ser Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu
305 310 315 320

Pro Thr Gly Met Leu Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr
325 330 335

Ser Arg Gln Gln Ile Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu
340 345 350

Ala Arg Gly Lys Ile Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr
355 360 365

Gly Gly Lys Ala Met Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr
370 375 380

Thr Val Ile Pro Arg Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala
385 390 395 400

Asn Ser Lys Gly Ser Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu
405 410 415

Cys Glu Ala Gly Leu Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu
420 425 430

Gly Met Asp Asn Ile Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro
435 440 445

Ser Ala Val Gln Glu Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly
450 455 460

Gly Asp Thr Gln Val Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn
465 470 475 480

Val Ser Ala Leu Ile Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu
485 490 495

Ile Arg Val Tyr Ala Leu Ser Gly Asp Gln Gly Gly Cys Ser Ser Ile
500 505 510

Leu Gly Asn Ser Lys His Lys Ala Pro Leu Ser Gly Pro His Ile Asn
515 520 525

Ala Ile Thr Glu Glu Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile
530 535 540

Pro Val Gln Glu Gln Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp
545 550 555 560

Lys Glu Arg Asp Ser Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr
565 570 575

Arg Val Ser Gln Asn Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val
580 585 590

Thr Tyr Val Leu Trp Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser

595 600 605

His Gly Asn Glu Arg Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met
610 615 620

Ala Phe Val Ala Pro Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile
625 630 635 640

Phe Ser Thr His Tyr Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala
645 650 655

Leu Arg Pro Gln Trp Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser
660 665 670

Thr Cys Ala Lys Lys Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro
675 680 685

Leu Asp Arg Leu Leu Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro
690 695 700

Leu Val Ile Ser Glu Val Leu His Gln Val Thr Pro Val Phe Arg His
705 710 715 720

Pro Pro Cys Ser Asn Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His
725 730 735

Gln Ala Ser Glu Lys Asp Met Met His Ser Ala Ser Ser Pro Pro Pro
740 745 750

Pro Arg Ala Leu Gln Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys
755 760 765

Val Leu Glu Ser Arg Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys
770 775 780

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Pro Trp Thr Val Leu Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr
785                 790                 795                 800

Leu Pro Ser Asn Ile Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala
                805                 810                 815

Asp Ser Leu Glu Glu Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe
            820                 825                 830

Pro Ser Ser Ser Leu His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu
        835                 840                 845

Thr Leu Asp Gln Leu Lys Met Arg Cys Asp Ser Leu Met Leu
    850                 855                 860
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: human T-cells

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: library 3 day PHA/pEF-BOS

(B) CLONE: human interleukin-12 receptor clone #5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 65..2050

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA        60

TCCG ATG GAG CCG CTG GTG ACC TGG GTG GTC CCC CTC CTC TTC CTC TTC        109
     Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe
       1               5                  10                  15

CTG CTG TCC AGG CAG GGC GCT GCC TGC AGA ACC AGT GAG TGC TGT TTT        157
Leu Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe
                20                  25                  30

CAG GAC CCG CCA TAT CCG GAT GCA GAC TCA GGC TCG GCC TCG GGC CCT        205
Gln Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro
            35                  40                  45

AGG GAC CTG AGA TGC TAT CGG ATA TCC AGT GAT CGT TAC GAG TGC TCC        253
Arg Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser
        50                  55                  60

TGG CAG TAT GAG GGT CCC ACA GCT GGG GTC AGC CAC TTC CTG CGG TGT        301
Trp Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys
    65                  70                  75

TGC CTT AGC TCC GGG CGC TGC TGC TAC TTC GCC GCC GGC TCA GCC ACC        349
Cys Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr
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80                  85                  90                  95

AGG CTG CAG TTC TCC GAC CAG GCT GGG GTG TCT GTG CTG TAC ACT GTC         397
Arg Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val
                    100                 105                 110

ACA CTC TGG GTG GAA TCC TGG GCC AGG AAC CAG ACA GAG AAG TCT CCT         445
Thr Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro
                115                 120                 125

GAG GTG ACC CTG CAG CTC TAC AAC TCA GTT AAA TAT GAG CCT CCT CTG         493
Glu Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu
            130                 135                 140

GGA GAC ATC AAG GTG TCC AAG TTG GCC GGG CAG CTG CGT ATG GAG TGG         541
Gly Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp
        145                 150                 155

GAG ACC CCG GAT AAC CAG GTT GGT GCT GAG GTG CAG TTC CGG CAC CGG         589
Glu Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg
160                 165                 170                 175

ACA CCC AGC AGC CCA TGG AAG TTG GGC GAC TGC GGA CCT CAG GAT GAT         637
Thr Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp
                    180                 185                 190

GAT ACT GAG TCC TGC CTC TGC CCC CTG GAG ATG AAT GTG GCC CAG GAA         685
Asp Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu
                195                 200                 205

TTC CAG CTC CGA CGA CGG CAG CTG GGG AGC CAA GGA AGT TCC TGG AGC         733
Phe Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser
            210                 215                 220
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AAG TGG AGC AGC CCC GTG TGC GTT CCC CCT GAA AAC CCC CCA CAG CCT        781
Lys Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro
    225                 230                 235

CAG GTG AGA TTC TCG GTG GAG CAG CTG GGC CAG GAT GGG AGG AGG CGG        829
Gln Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg
240                 245                 250                 255

CTG ACC CTG AAA GAG CAG CCA ACC CAG CTG GAG CTT CCA GAA GGC TGT        877
Leu Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys
                260                 265                 270

CAA GGG CTG GCG CCT GGC ACG GAG GTC ACT TAC CGA CTA CAG CTC CAC        925
Gln Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His
            275                 280                 285

ATG CTG TCC TGC CCG TGT AAG GCC AAG GCC ACC AGG ACC CTG CAC CTG        973
Met Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu
        290                 295                 300

GGG AAG ATG CCC TAT CTC TCG GGT GCT GCC TAC AAC GTG GCT GTC ATC       1021
Gly Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile
    305                 310                 315

TCC TCG AAC CAA TTT GGT CCT GGC CTG AAC CAG ACG TGG CAC ATT CCT       1069
Ser Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro
320                 325                 330                 335

GCC GAC ACC CAC ACA GAA CCA GTG GCT CTG AAT ATC AGC GTC GGA ACC       1117
Ala Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr
                340                 345                 350

AAC GGG ACC ACC ATG TAT TGG CCA GCC CGG GCT CAG AGC ATG ACG TAT       1165
Asn Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr
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                355                 360                 365

TGC ATT GAA TGG CAG CCT GTG GGC CAG GAC GGG GGC CTT GCC ACC TGC       1213
Cys Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys
            370                 375                 380

AGC CTG ACT GCG CCG CAA GAC CCG GAT CCG GCT GGA ATG GCA ACC TAC       1261
Ser Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr
        385                 390                 395

AGC TGG AGT CGA GAG TCT GGG GCA ATG GGG CAG GAA AAG TGT TAC TAC       1309
Ser Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr
400                 405                 410                 415

ATT ACC ATC TTT GCC TCT GCG CAC CCC GAG AAG CTC ACC TTG TGG TCT       1357
Ile Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser
                420                 425                 430

ACG GTC CTG TCC ACC TAC CAC TTT GGG GGC AAT GCC TCA GCA GCT GGG       1405
Thr Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly
                435                 440                 445

ACA CCG CAC CAC GTC TCG GTG AAG AAT CAT AGC TTG GAC TCT GTG TCT       1453
Thr Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser
            450                 455                 460

GTG GAC TGG GCA CCA TCC CTG CTG AGC ACC TGT CCC GGC GTC CTA AAG       1501
Val Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys
        465                 470                 475

GAG TAT GTT GTC CGC TGC CGA GAT GAA GAC AGC AAA CAG GTG TCA GAG       1549
Glu Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu
480                 485                 490                 495
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CAT CCC GTG CAG CCC ACA GAG ACC CAA GTT ACC CTC AGT GGC CTG CGG      1597
His Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg
                500                 505                 510

GCT GGT GTA GCC TAC ACG GTG CAG GTG CGA GCA GAC ACA GCG TGG CTG      1645
Ala Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu
            515                 520                 525

AGG GGT GTC TGG AGC CAG CCC CAG CGC TTC AGC ATC GAA GTG CAG GTT      1693
Arg Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val
        530                 535                 540

TCT GAT TGG CTC ATC TTC TTC GCC TCC CTG GGG AGC TTC CTG AGC ATC      1741
Ser Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile
    545                 550                 555

CTT CTC GTG GGC GTC CTT GGC TAC CTT GGC CTG AAC AGG GCC GCA CGG      1789
Leu Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg
560                 565                 570                 575

CAC CTG TGC CCG CCG CTG CCC ACA CCC TGT GCC AGC TCC GCC ATT GAG      1837
His Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu
                580                 585                 590

TTC CCT GGA GGG AAG GAG ACT TGG CAG TGG ATC AAC CCA GTG GAC TTC      1885
Phe Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe
            595                 600                 605

CAG GAA GAG GCA TCC CTG CAG GAG GCC CTG GTG GTA GAG ATG TCC TGG      1933
Gln Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp
        610                 615                 620

GAC AAA GGC GAG AGG ACT GAG CCT CTC GAG AAG ACA GAG CTA CCT GAG      1981
Asp Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu
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    625                 630                 635

GGT GCC CCT GAG CTG GCC CTG GAT ACA GAG TTG TCC TTG GAG GAT GGA      2029
Gly Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly
640                 645                 650                 655

GAC AGG TGC AAG GCC AAG ATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA         2080
Asp Arg Cys Lys Ala Lys Met
                660

CTCGCCCGAG GCTACGTAGC CTTT                                           2104
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 662 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
- (A) NAME/KEY: Region
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /note= "N-terminal signal peptide (1..20 or 23 or 24)"

(ix) FEATURE:
- (A) NAME/KEY: Region
- (B) LOCATION: 541..570
- (D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 571..662
(D) OTHER INFORMATION: /note= "cytoplasmic tail region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 52..64
(D) OTHER INFORMATION: /note= "sequence motif of cytokine receptor superfamily Cys52..Cys62SW"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 222..226
(D) OTHER INFORMATION: /note= "cytokine receptor superfamily motif (W222SKWS)"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 121..123
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 329..331
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 346..348
(D) OTHER INFORMATION: /note= "N-linked glycosylation

site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 352..354

(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 442..444

(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 456..458

(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 24..540

(D) OTHER INFORMATION: /note= "Extracellular region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe Leu
1               5                   10                  15

Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe Gln
            20                  25                  30
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Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg
        35                  40                  45

Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp
    50                  55                  60

Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys
65                  70                  75                  80

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg
                85                  90                  95

Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr
            100                 105                 110

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu
        115                 120                 125

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly
    130                 135                 140

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu
145                 150                 155                 160

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr
                165                 170                 175

Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp
            180                 185                 190

Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe
        195                 200                 205

Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys
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210 215 220

Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln
225 230 235 240

Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu
245 250 255

Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln
260 265 270

Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His Met
275 280 285

Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly
290 295 300

Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser
305 310 315 320

Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala
325 330 335

Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn
340 345 350

Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys
355 360 365

Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser
370 375 380

Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser
385 390 395 400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile
405 410 415

Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr
420 425 430

Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr
435 440 445

Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val
450 455 460

Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu
465 470 475 480

Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu His
485 490 495

Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala
500 505 510

Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg
515 520 525

Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser
530 535 540

Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu
545 550 555 560

Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His
565 570 575

Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe
580 585 590

Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln
595 600 605

Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp
610 615 620

Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly
625 630 635 640

Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp
645 650 655

Arg Cys Lys Ala Lys Met
660

Claims

1. A low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which

   (a) has low binding affinity for IL-12, and
   (b) when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12.

2. The protein of claim 1, wherein the IL-12 beta2 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1.

3. The protein of claim 2 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:2.

4. The protein of claim 3, wherein the IL-12 beta2 receptor protein has SEQ ID NO:2 or allelic forms or variants thereof.

5. The protein of any one of claims 1 to 4 encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined in any one of the preceding claims, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of Ig other than the first domain of said constant region.

6. A complex capable of binding to IL-12 with high affinity, comprising interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof as defined in any of claims 1 - 4 complexed with IL-12 beta1 receptor protein, or a fragment thereof which

   (a) has low binding affinity for IL-12, and
   (b) when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

7. The complex of claim 6, wherein the IL-12 beta1 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3.

8. The protein of claim 7 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:4.

9. The protein of claim 8, wherein the IL-12 beta1 receptor protein has SEQ ID NO:4 or allelic forms or variants thereof.

10. A protein encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of any one of claims 1 to 4 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a protein of any of claims 7 to 9 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.

11. A protein or complex of any one of claims 1 to 10 which is soluble.

12. Nucleic acids which encode a protein or complex of any one of claims 1 - 11.

13. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta2 receptor protein having the SEQ ID NO:1.

14. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta1 receptor protein having the SEQ ID NO:3.

15. A vector comprising a nucleic acid of any one of claims 12 to 14.

16. An expression vector comprising a nucleic acid of any one of claims 12 - 14 operably linked to control sequences recognised by a host cell.

17. A host cell transformed with a nucleic acid of any one of claims 12 to 16.

18. The host cell of claim 17 wherein the protein or complex is expressed on its surface.

19. The host cell of claim 18 wherein the host cell proliferates in the presence of IL-12.

20. The host cell of claims 17 - 19, wherein the host cell is transformed with a first vector comprising a nucleic acid encoding the protein as defined in claim 1 and a second vector comprising a nucleic acid encoding the protein as defined in claim 7 or with a single vector comprising a nucleic acid encoding the protein as defined in claim 1 and a nucleic acid encoding a protein as defined in claim 7.

21. An antibody directed against a protein of any of claims 1 to 11.

22. A process for the preparation of a protein of any of claims 1 to 11 which comprises the expression of a nucleic acid of any one of claims 12 to 14 in a suitable host cell.

23. A pharmaceutical composition comprising a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 and a pharmaceutically acceptable carrier.

24. The pharmaceutical composition of claim 23 which further comprises a therapeutically effective amount of one or more cytokine antagonists.

25. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament.

26. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament for the treatment of autoimmune dysfunction.

27. A method for screening compounds useful for inhibition of IL-12 activity, comprising

a) contacting a compound suspected of inhibiting IL-12 activity to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
b) detection of the inhibiting effect.

28. A method for screening compounds useful as agonists of IL-12, comprising

a) contacting a compound suspected of being an IL-12 agonist to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
b) detection of an agonist effect.

Fig. 1

10	20	30	40	50	60
TGCAGAGAAC	AGAGAAAGGA	CATCTGCGAG	GAAAGTTCCC	TGATGGCTGT	CAACAAAGTG
70	80	90	100	110	120
CCACGTCTCT	ATGGCTGTGT	ACGCTGAGCA	CACGATTTTA	TCGCGCCTAT	CATATCTTGG
130	140	150	160	170	180
TGCATAAACG	CACCTCACCT	CGGTCAACCC	TTGCTCCGTC	TTATGAGACA	GGCTTTATTA
190	200	210	220	230	240
TCCGCATTTT	ATATGAGGGG	AATCTGACGG	TGGAGAGAGA	ATTATCTTGC	TCAAGGCGAC
250	260	270	280	290	300
ACAGCAGAGC	CCACAGGTGG	CAGAATCCCA	CCCGAGCCCG	CTTCGACCCG	CGGGGTGGAA
310	320	330	340	350	360
ACCACGGGCG	CCCGCCCGGC	TGCGCTTCCA	GAGCTGAACT	GAGAAGCGAG	TCCTCTCCGC
370	380	390	400	410	420
CCTGCGGCCA	CCGCCCAGCC	CCGACCCCCG	CCCCGGCCCG	ATCCTCACTC	GCCGCCAGCT
430	440	450	460	470	480
CCCCGCGCCC	ACCCCGGAGT	TGGTGGCGCA	GAGGCGGGAG	GCGGAGGCGG	GAGGGCGGGC
490	500	510	520	530	540
GCTGGCACCG	GGAACGCCCG	AGCGCCGGCA	GAGAGCGCGG	AGAGCGCGAC	ACGTGCGGCC
550	560	570	580	590	600
CAGAGCACCG	GGGCCACCCG	GTCCCCGCAG	GCCCGGGACC	GCGCCCGCTG	GCAGGCGACA
610	620	630	640	650	660
CGTGGAAGAA	TACGGAGTTC	TATACCAGAG	TTGATTGTTG	ATGGCACATA	CTTTTAGAGG
670	680	690	700	710	720
ATGCTCATTG	GCATTTATGT	TTATAATCAC	GTGGCTGTTG	ATTAAAGCAA	AAATAGATGC
730	740	750	760	770	780
GTGCAAGAGA	GGCGATGTGA	CTGTGAAGCC	TTCCCATGTA	ATTTTACTTG	GATCCACTGT
790	800	810	820	830	840
CAATATTACA	TGCTCTTTGA	AGCCCAGACA	AGGCTGCTTT	CACTATTCCA	GACGTAACAA
850	860	870	880	890	900
GTTAATCCTG	TACAAGTTTG	ACAGAAGAAT	CAATTTTCAC	CATGGCCACT	CCCTCAATTC
910	920	930	940	950	960
TCAAGTCACA	GGTCTTCCCC	TTGGTACAAC	CTTGTTTGTC	TGCAAACTGG	CCTGTATCAA
970	980	990	1000	1010	1020
TAGTGATGAA	ATTCAAATAT	GTGGAGCAGA	GATCTTCGTT	GGTGTTGCTC	CAGAACAGCC

Fig. 1 CONT'D

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      1030       1040       1050       1060       1070       1080
TCAAAATTTA TCCTGCATAC AGAAGGGAGA ACAGGGGACT GTGGCCTGCA CCTGGGAAAG

      1090       1100       1110       1120       1130       1140
AGGACGAGAC ACCCACTTAT ACACTGAGTA TACTCTACAG CTAAGTGGAC CAAAAAATTT

      1150       1160       1170       1180       1190       1200
AACCTGGCAG AAGCAATGTA AAGACATTTA TTGTGACTAT TTGGACTTTG GAATCAACCT

      1210       1220       1230       1240       1250       1260
CACCCCTGAA TCACCTGAAT CCAATTTCAC AGCCAAGGTT ACTGCTGTCA ATAGTCTTGG

      1270       1280       1290       1300       1310       1320
AAGCTCCTCT TCACTTCCAT CCACATTCAC ATTCTTGGAC ATAGTGAGGC CTCTTCCTCC

      1330       1340       1350       1360       1370       1380
GTGGGACATT AGAATCAAAT TTCAAAAGGC TTCCGTGAGC AGATGTACCC TTTATTGGAG

      1390       1400       1410       1420       1430       1440
AGATGAGGGA CTGGTACTGC TTAATCGACT CAGATATCGG CCCAGTAACA GCAGGCTCTG

      1450       1460       1470       1480       1490       1500
GAATATGGTT AATGTTACAA AGGCCAAAGG AAGACATGAT TTGCTGGATC TGAAACCATT

      1510       1520       1530       1540       1550       1560
TACAGAATAT GAATTTCAGA TTTCCTCTAA GCTACATCTT TATAAGGGAA GTTGGAGTGA

      1570       1580       1590       1600       1610       1620
TTGGAGTGAA TCATTGAGAG CACAAACACC AGAAGAAGAG CCTACTGGGA TGTTAGATGT

      1630       1640       1650       1660       1670       1680
CTGGTACATG AAACGGCACA TTGACTACAG TAGACAACAG ATTTCTCTTT TCTGGAAGAA

      1690       1700       1710       1720       1730       1740
TCTGAGTGTC TCAGAGGCAA GAGGAAAAAT TCTCCACTAT CAGGTGACCT TGCAGGAGCT

      1750       1760       1770       1780       1790       1800
GACAGGAGGG AAAGCCATGA CACAGAACAT CACAGGACAC ACCTCCTGGA CCACAGTCAT

      1810       1820       1830       1840       1850       1860
TCCTAGAACC GGAAATTGGG CTGTGGCTGT GTCTGCAGCA AATTCAAAAG GCAGTTCTCT

      1870       1880       1890       1900       1910       1920
GCCCACTCGT ATTAACATAA TGAACCTGTG TGAGGCAGGG TTGCTGGCTC CTCGCCAGGT

      1930       1940       1950       1960       1970       1980
CTCTGCAAAC TCAGAGGGCA TGGACAACAT TCTGGTGACT TGGCAGCCTC CCAGGAAAGA

      1990       2000       2010       2020       2030       2040
TCCCTCTGCT GTTCAGGAGT ACGTGGTGGA ATGGAGAGAG CTCCATCCAG GGGGTGACAC
```

Fig. 1 CONT'D

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      2050       2060       2070       2080       2090       2100
ACAGGTCCCT CTAAACTGGC TACGGAGTCG ACCCTACAAT GTGTCTGCTC TGATTTCAGA

      2110       2120       2130       2140       2150       2160
GAACATAAAA TCCTACATCT GTTATGAAAT CCGTGTGTAT GCACTCTCAG GGGATCAAGG

      2170       2180       2190       2200       2210       2220
AGGATGCAGC TCCATCCTGG GTAACTCTAA GCACAAAGCA CCACTGAGTG GCCCCCACAT

      2230       2240       2250       2260       2270       2280
TAATGCCATC ACAGAGGAAA AGGGGAGCAT TTTAATTTCA TGGAACAGCA TTCCAGTCCA

      2290       2300       2310       2320       2330       2340
GGAGCAAATG GGCTGCCTCC TCCATTATAG GATATACTGG AAGGAACGGG ACTCCAACTC

      2350       2360       2370       2380       2390       2400
CCAGCCTCAG CTCTGTGAAA TTCCCTACAG AGTCTCCCAA AATTCACATC CAATAAACAG

      2410       2420       2430       2440       2450       2460
CCTGCAGCCC CGAGTGACAT ATGTCCTGTG GATGACAGCT CTGACAGCTG CTGGTGAAAG

      2470       2480       2490       2500       2510       2520
TTCCCACGGA AATGAGAGGG AATTTTGTCT GCAAGGTAAA GCCAATTGGA TGGCGTTTGT

      2530       2540       2550       2560       2570       2580
GGCACCAAGC ATTTGCATTG CTATCATCAT GGTGGGCATT TTCTCAACGC ATTACTTCCA

      2590       2600       2610       2620       2630       2640
GCAAAAGGTG TTTGTTCTCC TAGCAGCCCT CAGACCTCAG TGGTGTAGCA GAGAAATTCC

      2650       2660       2670       2680       2690       2700
AGATCCAGCA AATAGCACTT GCGCTAAGAA ATATCCCATT GCAGAGGAGA AGACACAGCT

      2710       2720       2730       2740       2750       2760
GCCCTTGGAC AGGCTCCTGA TAGACTGGCC CACGCCTGAA GATCCTGAAC CGCTGGTCAT

      2770       2780       2790       2800       2810       2820
CAGTGAAGTC CTTCATCAAG TGACCCCAGT TTTCAGACAT CCCCCCTGCT CCAACTGGCC

      2830       2840       2850       2860       2870       2880
ACAAAGGGAA AAAGGAATCC AAGGTCATCA GGCCTCTGAG AAAGACATGA TGCACAGTGC

      2890       2900       2910       2920       2930       2940
CTCAAGCCCA CCACCTCCAA GAGCTCTCCA AGCTGAGAGC AGACAACTGG TGGATCTGTA

      2950       2960       2970       2980       2990       3000
CAAGGTGCTG GAGAGCAGGG GCTCCGACCC AAAGCCAGAA AACCCAGCCT GTCCCTGGAC

      3010       3020       3030       3040       3050       3060
GGTGCTCCCA GCAGGTGACC TTCCCACCCA TGATGGCTAC TTACCCTCCA ACATAGATGA
```

Fig. 1 CONT'D

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      3070       3080       3090       3100       3110       3120
CCTCCCCTCA CATGAGGCAC CTCTCGCTGA CTCTCTGGAA GAACTGGAGC CTCAGCACAT

      3130       3140       3150       3160       3170       3180
CTCCCTTTCT GTTTTCCCCT CAAGTTCTCT TCACCCACTC ACCTTCTCCT GTGGTGATAA

      3190       3200       3210       3220       3230       3240
GCTGACTCTG GATCAGTTAA AGATGAGGTG TGACTCCCTC ATGCTCTGAG TGGTGAGGCT

      3250       3260       3270       3280       3290       3300
TCAAGCCTTA AAGTCAGTGT GCCCTCAACC AGCACAGCCT GCCCCAATTC CCCCAGCCCC

      3310       3320       3330       3340       3350       3360
TGCTCCAGCA GCTGTCATCT CTGGGTGCCA CCATCGGTCT GGCTGCAGCT AGAGGACAGG

      3370       3380       3390       3400       3410       3420
CAAGCCAGCT CTGGGGGAGT CTTAGGAACT GGGAGTTGGT CTTCACTCAG ATGCCTCATC

      3430       3440       3450       3460       3470       3480
TTGCCTTTCC CAGGGCCTTA AAATTACATC CTTCACTGTG TGGACCTAGA GACTCCAACT

      3490       3500       3510       3520       3530       3540
TGAATTCCTA GTAACTTTCT TGGTATGCTG GCCAGAAAGG GAAATGAGGA GGAGAGTAGA

      3550       3560       3570       3580       3590       3600
AACCACAGCT CTTAGTAGTA ATGGCATACA GTCTAGAGGA CCATTCATGC AATGACTATT

      3610       3620       3630       3640       3650       3660
TCTAAAGCAC CTGCTACACA GCAGGCTGTA CACAGCAGAT CAGTACTGTT CAACAGAACT

      3670       3680       3690       3700       3710       3720
TCCTGAGATG ATGGAAATGT TCTACCTCTG CACTCACTGT CCAGTACATT AGACACTAGG

      3730       3740       3750       3760       3770       3780
CACATTGGCT GTTAATCACT TGGAATGTGT TTAGCTTGAC TGAGGAATTA AATTTTGATT

      3790       3800       3810       3820       3830       3840
GTAAATTTAA ATCGCCACAC ATGGCTAGTG GCTACTGTAT TGGAGTGCAC AGCTCTAGAT

      3850       3860       3870       3880       3890       3900
GGCTCCTAGA TTATTGAGAG CCTCCAAAAC AAATCAACCT AGTTCTATAG ATGAAGACAT

      3910       3920       3930       3940       3950       3960
AAAAGACACT GGTAAACACC AATGTAAAAG GGCCCCCAAG GTGGTCATGA CTGGTCTCAT

      3970       3980       3990       4000       4010       4020
TTGCAGAAGT CTAAGAATGT ACCTTTTTCT GGCCGGGCGT GGTAGCTCAT GCCTGTAATC

      4030       4040
CCAGCACTTT GGGAGGCTGA
```

Fig. 2

```
  1 MAHTFRGCSL AFMFIITWLL IKAKIDACKR GDVTVKPSHV ILLGSTVNIT
 51 CSLKPRQGCF HYSRRNKLIL YKFDRRINFH HGHSLNSQVT GLPLGTTLFV
101 CKLACINSDE IQICGAEIFV GVAPEQPQNL SCIQKGEQGT VACTWERGRD
151 THLYTEYTLQ LSGPKNLTWQ KQCKDIYCDY LDFGINLTPE SPESNFTAKV
201 TAVNSLGSSS SLPSTFTFLD IVRPLPPWDI RIKFQKASVS RCTLYWRDEG
251 LVLLNRLRYR PSNSRLWNMV NVTKAKGRHD LLDLKPFTEY EFQISSKLHL
301 YKGSWSDWSE SLRAQTPEEE PTGMLDVWYM KRHIDYSRQQ ISLFWKNLSV
351 SEARGKILHY QVTLQELTGG KAMTQNITGH TSWTTVIPRT GNWAVAVSAA
401 NSKGSSLPTR INIMNLCEAG LLAPRQVSAN SEGMDNILVT WQPPRKDPSA
451 VQEYVVEWRE LHPGGDTQVP LNWLRSRPYN VSALISENIK SYICYEIRVY
501 ALSGDQGGCS SILGNSKHKA PLSGPHINAI TEEKGSILIS WNSIPVQEQM
551 GCLLHYRIYW KERDSNSQPQ LCEIPYRVSQ NSHPINSLQP RVTYVLWMTA
601 LTAAGESSHG NEREFCLQGK ANWMAFVAPS ICIAIIMVGI FSTHYFQQKV
651 FVLLAALRPQ WCSREIPDPA NSTCAKKYPI AEEKTQLPLD RLLIDWPTPE
701 DPEPLVISEV LHQVTPVFRH PPCSNWPQRE KGIQGHQASE KDMMHSASSP
751 PPPRALQAES RQLVDLYKVL ESRGSDPKPE NPACPWTVLP AGDLPTHDGY
801 LPSNIDDLPS HEAPLADSLE ELEPQHISLS VFPSSSLHPL TFSCGDKLTL
851 DQLKMRCDSL ML
```

Fig. 3

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        10         20         30         40         50         60         70
GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGC TCTACGTGGA TCCGATGGAG

        80         90        100        110        120        130        140
CCGCTGGTGA CCTGGGTGGT CCCCCTCCTC TTCCTCTTCC TGCTGTCCAG GCAGGGCGCT GCCTGCAGAA

       150        160        170        180        190        200        210
CCAGTGAGTG CTGTTTTCAG GACCCGCCAT ATCCGGATGC AGACTCAGGC TCGGCCTCGG GCCCTAGGGA

       220        230        240        250        260        270        280
CCTGAGATGC TATCGGATAT CCAGTGATCG TTACGAGTGC TCCTGGCAGT ATGAGGGTCC CACAGCTGGG

       290        300        310        320        330        340        350
GTCAGCCACT TCCTGCGGTG TTGCCTTAGC TCCGGGCGCT GCTGCTACTT CGCCGCCGGC TCAGCCACCA

       360        370        380        390        400        410        420
GGCTGCAGTT CTCCGACCAG GCTGGGGTGT CTGTGCTGTA CACTGTCACA CTCTGGGTGG AATCCTGGGC

       430        440        450        460        470        480        490
CAGGAACCAG ACAGAGAAGT CTCCTGAGGT GACCCTGCAG CTCTACAACT CAGTTAAATA TGAGCCTCCT

       500        510        520        530        540        550        560
CTGGGAGACA TCAAGGTGTC CAAGTTGGCC GGGCAGCTGC GTATGGAGTG GGAGACCCCG GATAACCAGG

       570        580        590        600        610        620        630
TTGGTGCTGA GGTGCAGTTC CGGCACCGGA CACCCAGCAG CCCATGGAAG TTGGGCGACT GCGGACCTCA

       640        650        660        670        680        690        700
GGATGATGAT ACTGAGTCCT GCCTCTGCCC CCTGGAGATG AATGTGGCCC AGGAATTCCA GCTCCGACGA

       710        720        730        740        750        760        770
CGGCAGCTGG GGAGCCAAGG AAGTTCCTGG AGCAAGTGGA GCAGCCCCGT GTGCGTTCCC CCTGAAAACC

       780        790        800        810        820        830        840
CCCCACAGCC TCAGGTGAGA TTCTCGGTGG AGCAGCTGGG CCAGGATGGG AGGAGGCGGC TGACCCTGAA

       850        860        870        880        890        900        910
AGAGCAGCCA ACCCAGCTGG AGCTTCCAGA AGGCTGTCAA GGGCTGGCGC CTGGCACGGA GGTCACTTAC

       920        930        940        950        960        970        980
CGACTACAGC TCCACATGCT GTCCTGCCCG TGTAAGGCCA AGGCCACCAG GACCCTGCAC CTGGGGAAGA

       990       1000       1010       1020       1030       1040       1050
TGCCCTATCT CTCGGGTGCT GCCTACAACG TGGCTGTCAT CTCCTCGAAC CAATTTGGTC CTGGCCTGAA

      1060       1070       1080       1090       1100       1110       1120
CCAGACGTGG CACATTCCTG CCGACACCCA CACAGAACCA GTGGCTCTGA ATATCAGCGT CGGAACCAAC
```

Fig. 3 CONT'D

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      1130       1140       1150       1160       1170       1180       1190
GGGACCACCA TGTATTGGCC AGCCCGGGCT CAGAGCATGA CGTATTGCAT TGAATGGCAG CCTGTGGGCC

      1200       1210       1220       1230       1240       1250       1260
AGGACGGGGG CCTTGCCACC TGCAGCCTGA CTGCGCCGCA AGACCCGGAT CCGGCTGGAA TGGCAACCTA

      1270       1280       1290       1300       1310       1320       1330
CAGCTGGAGT CGAGAGTCTG GGGCAATGGG GCAGGAAAAG TGTTACTACA TTACCATCTT TGCCTCTGCG

      1340       1350       1360       1370       1380       1390       1400
CACCCCGAGA AGCTCACCTT GTGGTCTACG GTCCTGTCCA CCTACCACTT TGGGGGCAAT GCCTCAGCAG

      1410       1420       1430       1440       1450       1460       1470
CTGGGACACC GCACCACGTC TCGGTGAAGA ATCATAGCTT GGACTCTGTG TCTGTGGACT GGGCACCATC

      1480       1490       1500       1510       1520       1530       1540
CCTGCTGAGC ACCTGTCCCG GCGTCCTAAA GGAGTATGTT GTCCGCTGCC GAGATGAAGA CAGCAAACAG

      1550       1560       1570       1580       1590       1600       1610
GTGTCAGAGC ATCCCGTGCA GCCCACAGAG ACCCAAGTTA CCCTCAGTGG CCTGCGGGCT GGTGTAGCCT

      1620       1630       1640       1650       1660       1670       1680
ACACGGTGCA GGTGCGAGCA GACACAGCGT GGCTGAGGGG TGTCTGGAGC CAGCCCCAGC GCTTCAGCAT

      1690       1700       1710       1720       1730       1740       1750
CGAAGTGCAG GTTTCTGATT GGCTCATCTT CTTCGCCTCC CTGGGGAGCT TCCTGAGCAT CCTTCTCGTG

      1760       1770       1780       1790       1800       1810       1820
GGCGTCCTTG GCTACCTTGG CCTGAACAGG GCCGCACGGC ACCTGTGCCC GCCGCTGCCC ACACCCTGTG

      1830       1840       1850       1860       1870       1880       1890
CCAGCTCCGC CATTGAGTTC CCTGGAGGGA AGGAGACTTG GCAGTGGATC AACCCAGTGG ACTTCCAGGA

      1900       1910       1920       1930       1940       1950       1960
AGAGGCATCC CTGCAGGAGG CCCTGGTGGT AGAGATGTCC TGGGACAAAG GCGAGAGGAC TGAGCCTCTC

      1970       1980       1990       2000       2010       2020       2030
GAGAAGACAG AGCTACCTGA GGGTGCCCCT GAGCTGGCCC TGGATACAGA GTTGTCCTTG GAGGATGGAG

      2040       2050       2060       2070       2080       2090       2100
ACAGGTGCAA GGCCAAGATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA CTCGCCCGAG GCTACGTAGC
```

Fig. 4

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        10         20         30         40         50         60         70
MEPLVTWVVP LLFLFLLSRQ GAACRTSECC FQDPPYPDAD SGSASGPRDL RCYRISSDRY ECSWQYEGPT

        80         90        100        110        120        130        140
AGVSHFLRCC LSSGRCCYFA AGSATRLQFS DQAGVSVLYT VTLWVESWAR NQTEKSPEVT LQLYNSVKYE

       150        160        170        180        190        200        210
PPLGDIKVSK LAGQLRMEWE TPDNQVGAEV QFRHRTPSSP WKLGDCGPQD DDTESCLCPL EMNVAQEFQL

       220        230        240        250        260        270        280
RRRQLGSQGS SWSKWSSPVC VPPENPPQPQ VRFSVEQLGQ DGRRRLTLKE QPTQLELPEG CQGLAPGTEV

       290        300        310        320        330        340        350
TYRLQLHMLS CPCKAKATRT LHLGKMPYLS GAAYNVAVIS SNQFGPGLNQ TWHIPADTHT EPVALNISVG

       360        370        380        390        400        410        420
TNGTTMYWPA RAQSMTYCIE WQPVGQDGGL ATCSLTAPQD PDPAGMATYS WSRESGAMGQ EKCYYITIFA

       430        440        450        460        470        480          4
SAHPEKLTLW STVLSTYHFG GNASAAGTPH HVSVKNHSLD SVSVDWAPSL LSTCPGVLKE YVVRCRDEDS

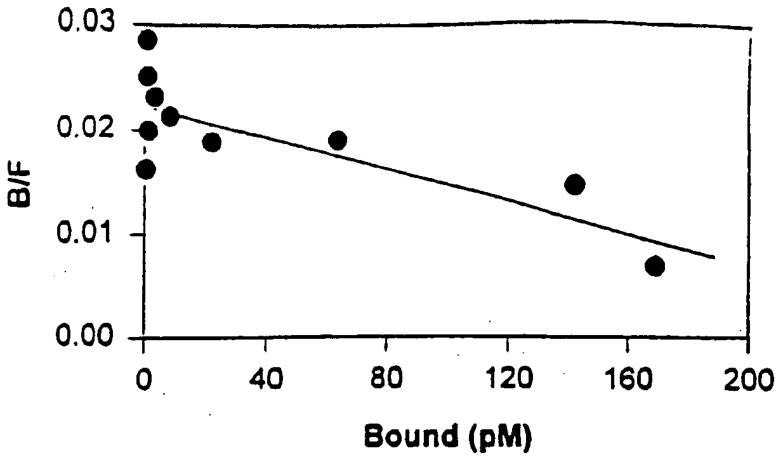
       500        510        520        530        540        550        560
KQVSEHPVQP TETQVTLSGL RAGVAYTVQV RADTAWLRGV WSQPQRFSIE VQVSDWLIFF ASLGSFLSIL

       570        580        590        600        610        620        630
LVGVLGYLGL NRAARHLCPP LPTPCASSAI EFPGGKETWQ WINPVDFQEE ASLQEALVVE MSWDKGERTE

       640        650        660
PLEKTELPEG APELALDTEL SLEDGDRCKA KM
```

Fig. 5

A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80
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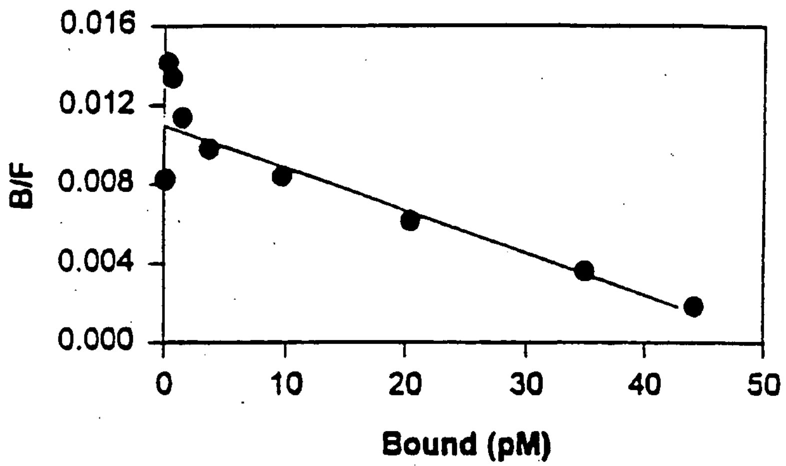
160
200
Bound (pM)

B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0
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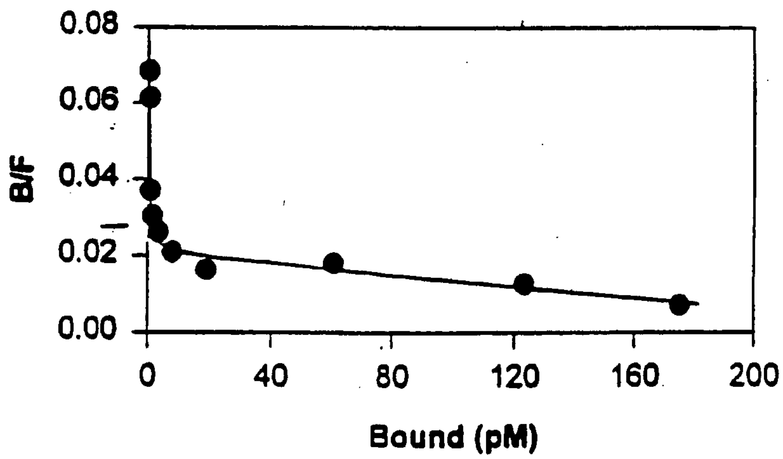
20
30
40
50
Bound (pM)

C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B/F
0
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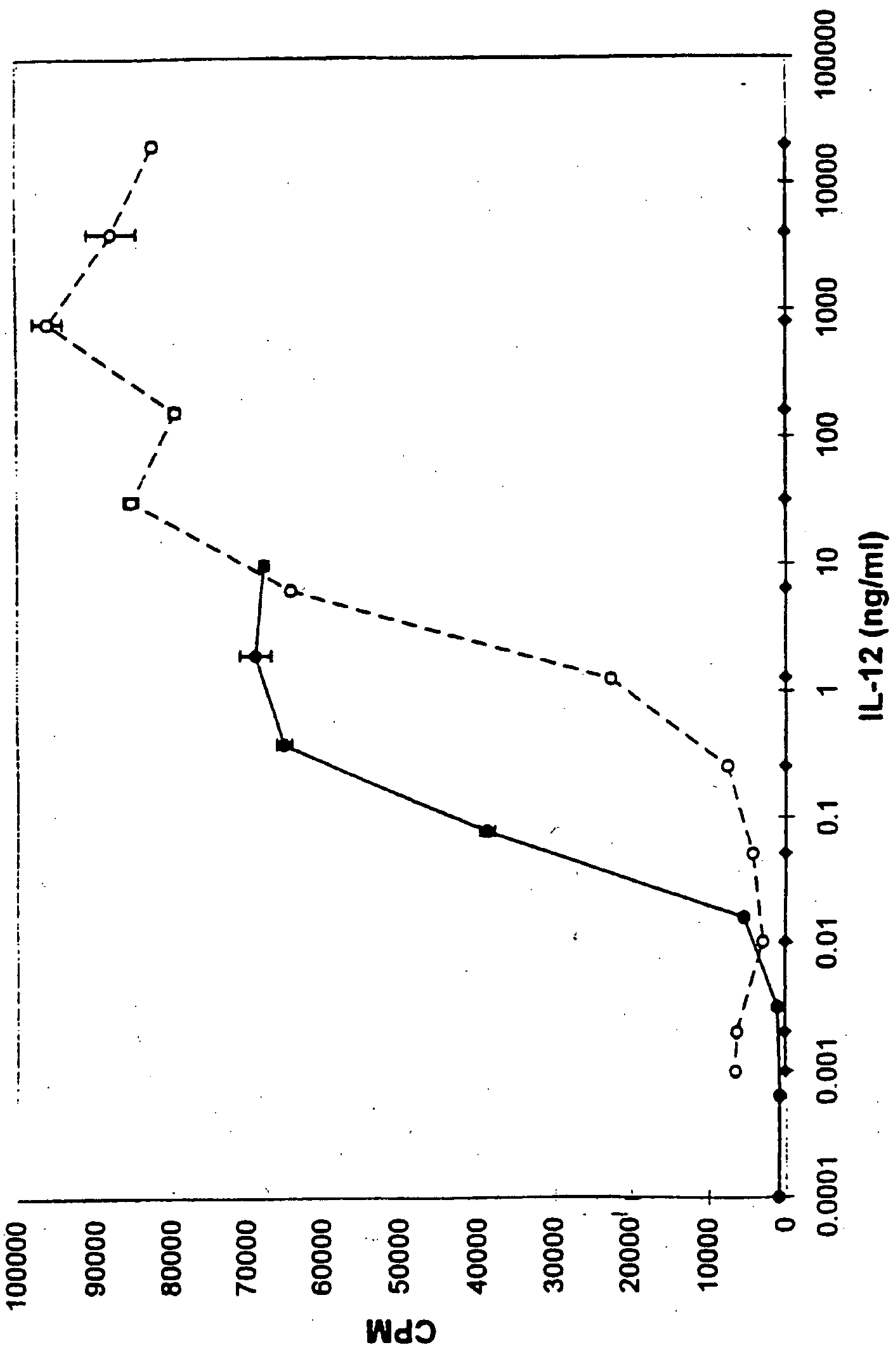
80
120
160
200
Bound (pM)

Fig. 6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60000
50000
40000
30000
20000
10000
0
IL-12 (ng/ml)
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
100000